IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Maurer et al. Application No.: 10/019,199 Filed: 12/20/2001 Title: Methods for Preparation of Lipid-Encapsulated Therapeutic Agents Attorney Docket No.: INEX.P-005-USNP	Group Art Unit: 1615 Examiner: Kishore, Gollamudi S.

TRANSMITTAL LETTER

Applicants submit herewith the documents mentioned below:

Brief for Appellant and Appendix (in triplicate)

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Respectfully submitted,

Carl Oppedahl
PTO Registration No. 32,746
PO Box 5068
Dillon, CO 80435-5068
Tel: 970-468-6600 / Fax: 970-468-0104

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Application No.: 10/019,199	
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Title: Methods for Preparation of Lipid-Encapsulated Therapeutic Agents	Examiner: Kishore, Gollamudi S.
Attorney Docket No.: INEX.P-005	

BRIEF FOR APPELLANT

This brief is filed in support of Applicants' Appeal from the final rejection mailed 12/30/2003. Consideration of the application and reversal of the rejections are respectfully urged.

Real Party in Interest

The real party in interest is Inex Pharmaceuticals Corp.

Related Appeals and Interferences

To Applicants' knowledge, there are no related Appeals or Interferences.

Status of Claims

Claims 1-12 have been canceled. Claims 13-32 are pending and are the subject of this appeal. No other claims have been presented.

Status of Amendments

All amendments have been entered.

Summary of Invention

Preparation of lipid-encapsulated therapeutic agents has stringent requirements if the agents are to be used as pharmaceuticals. They should have compositions within a defined range of tolerances as it relates to amount of therapeutic encapsulated, consistent size of particles, and consistent degrees of encapsulation. In the case of charged therapeutic agents and charged lipids, lipid particles which meet these requirements are known, as well as bench scale methods for making them. (Specification, pages 1-2.) These methods, however, are not suitable for large-scale manufacture of lipid-encapsulated therapeutic agents for actual pharmaceuticals. Methods which do scale up well are not suitable for use with charged therapeutics and oppositely charged lipids. (Specification, Page 3.)

The present invention relates to a method for preparing fully lipid-encapsulated therapeutic agent particles of a charged therapeutic agent and an oppositely charged lipid. As set forth in independent claim 13, this method involves the steps of:

- combining a lipid composition comprising preformed lipid vesicles, a charged therapeutic agent, and a destabilizing agent to form a mixture of preformed vesicles and therapeutic agent in a destabilizing solvent, wherein said
- destabilizing solvent is effective to destabilize the membrane of the preformed lipid vesicles without disrupting the vesicles,
- incubating the mixture for a period of time sufficient to allow the encapsulation of the therapeutic agent within the preformed lipid vesicles, and
- removing the destabilizing agent.

The preformed lipid vesicles comprise a charged lipid which has a charge which is opposite to the charge of the charged therapeutic agent and a modified lipid having a steric barrier moiety for control of aggregation. The amount of modified lipid is further defined in the claims as being present in the preformed vesicles in an amount effective to retard, but not prevent, aggregation of the preformed vesicles. This method, due to its simplicity, can be readily scaled up, and therefore answers the need for an effective means to prepare commercially relevant amounts of lipid-encapsulated charged therapeutic agent.

Issues on Appeal

1. Are the pending claims unpatentable under 35 USC § 103 as obvious over the combination of US Patent No. 6,447,800 of Hope in view of US Patent No. 5,976,567 of Wheeler or WO 98/51278?

2. Are the pending claims unpatentable under 35 USC § 103 as obvious over the combination of US Patent No. 6,447,800 of Hope in view of Malone and US Patent No. 6,365,179 of Zalipsky?

3. Are claims 13-20 and 25-32 claims unpatentable under 35 USC § 103 as obvious over the combination of Schubert in view of Malone and either Zalipsky or WO 98/51278?

Applicants submit that all of these questions should be answered in the negative, and that the rejections of the Examiner should be reversed.

Grouping of Claims

All claims are argued as a single group and stand or fall together, except that claims 21-24 which are not rejected over the combination based on Schubert do not stand or fall with the rejected claims on this issue.

Argument

Legal Standards for Obviousness Rejections

In considering a rejection under 35 USC § 103, it must be remembered that "obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion or incentive supporting the combination." *Carella v. Starlight Archery and Pro Line Co.*, 804 F.2d 135, 140, 231 USPQ 644, 647 (Fed. Cir. 1986) (citing *ACS Hosp. Syss., Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)). "[T]he factual inquiry whether to combine references must be thorough and searching." *McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1351-52, 60 USPQ2d 1001, 1008 (Fed. Cir. 2001). This factual question cannot "be resolved on subjective belief and unknown

authority," *In re Lee*, 277 F.3d 1338, 1343-44, 61 USPQ2d 1430, 1434 (Fed. Cir. 2002); "it must be based on objective evidence of record." Id. at 1343, 61 USPQ2d at 1434.

The U.S. Court of Appeals for the Federal Circuit has stated that "[t]he mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggested the desirability of the modification." *In re Fritch*, 972 F.2d 1260, 1266, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992) (*citing In re Gordon,* 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Cir. 1984)). Although this statement is couched in terms of modifying the prior art, it is equally applicable to combining teachings found in the prior art. Specifically, the mere fact that teachings found in the prior art could be combined as proposed by an examiner does not make the combination obvious "absent some teaching, suggestion or incentive supporting the combination." *Carella*, 804 F.2d at 140, 231 USPQ at 647 (citing *ACS Hosp. Syss., Inc.*, 732 F.2d at 1577, 221 USPQ at 933). Stated differently, "citing references which merely indicate the isolated elements ... are known is not a sufficient basis for concluding that the combination of elements would have been obvious." *Ex Parte Hiyamizu*, 10 USPQ 2d 1393, 1394 (POBAI 1988).

The Claims Are Patentable over Hope in view of Wheeler or WO 98/51278

The Examiner rejected claims 12-32 under 35 USC § 103 as obvious over the combination of US Patent No. 6,447,800 of Hope in view of US Patent No. 5,976,567 of Wheeler or WO 98/51278. In support of their arguments against this rejection, Applicants submitted a declaration of Dr. Michael Hope, who is an inventor on all three of the references cited in this rejection, as well as a person of considerable skill in the art. This declaration is referenced in this brief as "(Hope, ¶ ___)", and consideration of this paper of record is requested and encouraged. The Board should be aware that Dr. Hope is employed by the assignee of this application, Inex Pharmaceuticals Corporation.

Independent claims 13 contains the following limitations:

(1) combining a lipid composition comprising preformed lipid vesicles, a charged therapeutic agent, and a destabilizing agent to form a mixture of preformed

vesicles and therapeutic agent in a destabilizing solvent, wherein said destabilizing solvent is effective to destabilize the membrane of the preformed lipid vesicles without disrupting the vesicles,

(2) incubating the mixture for a period of time sufficient to allow the encapsulation of the therapeutic agent within the preformed lipid vesicles, and

(3) removing the destabilizing agent,

(4) the preformed lipid vesicles comprise a charged lipid which has a charge which is opposite to the charge of the charged therapeutic agent and a modified lipid having a steric barrier moiety for control of aggregation,

(5) wherein the modified lipid is present in the preformed vesicles in an amount effective to retard, but not prevent, aggregation of the preformed vesicles.

The Examiner asserts that Hope teaches

> a method of preparation of liposomes containing a variety of active agents. The method involves combining already formed liposomes with an active agent and organic solvent, ethanol (at least 10%), allowing a certain amount of time and diluting the organic solvent in the external phase. The presence of the external solvent according to Hope increases the permeability of the membrane (without disrupting the liposomes) and when the organic solvent is diluted, the permeability decreases.

(Office Action of 12/30/2003, Page 2.) The Examiner states that the differences between the Hope disclosure and the claimed invention is (1) the use of a cationic lipid and a teaching of removal of the organic solvent. As a first matter, Applicants submit that list of differences is incomplete.

The therapeutic agents used in the method of the present invention are charged. The Hope patent states that "generally highly negatively charged species such as polynucleotides do not cross the liposomal membranes permeabilized by the solvent technique disclosed herein." (Col. 10, lines 7-9). (Hope, ¶ 12). Indeed, based on experiments conducted in 1990-1992 by Dr. Hope, the technique disclosed in the Hope patent does not work with charged oligonucleotides. (Hope, ¶ 11).

The Examiner chooses to ignore both the express statement in the reference and the declaration from its author based upon a statement in the application (Col. 10, lines 10-13) that acknowledges that charged species can be rendered less charged, or uncharged by adjustment of the pH or by chemical modification. From this, the Examiner infers, without evidence or even reasoned argument, that the Hope method is taught to work for at least some charged species. Because of this, he asserts that Hope teaches this aspect of the invention. The phrase in the Hope patent however, "conversion to less highly charged analogs" does not necessarily imply that charged species will cross the permeabilized membrane. It is therefore improper for the Examiner to adopt this understanding of the language based on the teaching of the present application.

The Examiner acknowledges that Hope does not teach a charged lipid as required by the present claims. To address this deficiency of the Hope reference, he cites Wheeler. Wheeler is different from Hope in several important respects which make the combination of these references inappropriate.

The key to the Hope patent is that ethanol permeabilizes the membrane without changing the structure. The ethanol establishes an organic-solvent induced permeation gradient (col 8, lines 34-65). The present claims require a destabilizing solvent that "is effective to destabilize the membrane of the preformed lipid vesicles **without disrupting the vesicles**." In contrast, the nucleic acid loading method disclosed in Wheeler restructures the lipid material by **dissolving it in a solvent**. (Hope, ¶ 9.) The Hope and Wheeler methods both make use of ethanol, but this is not a point of similarity, because the ethanol in Wheeler is not used to permeabilize a pre-formed lipid membrane. There is no disclosure in Wheeler of introducing nucleic acids, or any other charged material through the membrane of a pre-formed lipid particle. Instead, as reflected in Fig. 40 of Wheeler, formation of the particles results from rearrangement of the lipid membrane or from a coating of particles onto the DNA. (Hope, ¶ 13.)

The Examiner acknowledges these differences, noting that in Wheeler "preformed liposomes are not used." (Office Action of 12/30/2003, Page 3.) Nevertheless, the Examiner asserts that "the use of cationic lipids in the method of Hope, if the active agent involves a

nucleic acid would have been obvious ... since Wheeler teaches that cationic lipids are efficient in transfecting cells." (Id.) Applicants submit that the motivation for this combination can be found nowhere in the cited art, and therefore can only be the result of improper reliance on the present invention as a guide.

The only reason offered by the Examiner for putting the cationic lipids into the formulation of Hope is because of the utility of cationic lipids for transfection. However, there are other reasons besides transfection for placing therapeutic agents, including oligonucleotides, into lipid carriers, and Hope does not mention the use of these particles for transfection. Thus, the Examiner's sole reason for combining the references is a utility which they have not been shown to share.

Furthermore, the argument offered by the Examiner that adding cationic lipids, and therefore positive charges to the membrane would be expected to enhance the passage of negatively charged therapeutic agents through the membrane does not make sense. One thing Wheeler clearly teaches is the fact that positively charged lipids and negatively charged nucleic acids stick together and form complexes. The person skilled in the art also knows from the Hope patent that negatively-charged species do not pass through an uncharged membrane. Why then would a person skilled in the art imagine that negatively charged polynucleotides would pass through a membrane better, when that membrane contains positive charges to which they can stick as shown in Wheeler? Applicants submit that, "without knowledge of the present invention, the idea of adding charged lipids of opposite charge to the therapeutic agent would not be a reasonable option, since one skilled in the art would reasonably expect that adding positive charges in the lipid would cause negatively charged lipid to bind to the lipid, making passage through the membrane more, not less, difficult." (Declaration ¶ 12).

From these explanations, it can be seen that the basis for the combination of the Hope and Wheeler reference is really based only on the superficial similarity in words and materials, and not on a real similarity which would provide motivation to a person skilled in the art. The types of lipid structures and the physical changes that the lipid structures undergo in the two applications are different. One involves transport of materials through an uncharged membrane,

while the other involves formation of complex based on interactions of charged species. (Hope, ¶¶ 9 and 16) Further, the role of ethanol in the two references is different. (Hope, ¶ 13). The purposes of the lipid structures in the Hope and the Wheeler references are different. (Hope, ¶ 15) Thus, it can be seen that the rejection is based solely on finding the isolated elements, and that the motivation to make the combination is lacking. This is not enough, since "citing references which merely indicate the isolated elements ... are known is not a sufficient basis for concluding that the combination of elements would have been obvious." *Ex Parte Hiyamizu*, 10 USPQ 2d 1393, 1394 (POBAI 1988). For this reason, as stated in the declaration (Hope, ¶ 8), Dr. Hope, as both an author of the cited references and a person skilled in the art, "believe[s] that the Examiner is taking selected parts from each of these references in a manner which would not be apparent absent the guidance of the present application." This is an improper basis for a rejection under 35 USC § 103.

Furthermore, the claims also contain a requirement for a modified lipid that acts as a steric barrier, and specifies that the amount of this lipid is amount effective to retard, but not prevent, aggregation of the preformed vesicles. Hope does not disclose the use of such lipids, and this therefore is another difference between Hope and the claimed invention. To address this deficiency of the primary reference, that the Examiner has said that the addition of PEG-lipids to the compositions of Hope would have been obvious since WO 98/51278 teaches their ability to provide steric stabilization. Again, this is an instance where the Examiner has improperly used the invention to pick elements from the art, without showing any reason why this would have been a reasonable option to the person skilled in the art without reliance on Applicants' invention.

For example, the Examiner has not explained why one skilled in the art would think that "steric stablization" is needed in the liposomes of Hope. Steric stabilization is used in the reference to reduce or eliminate aggregation of lipid particles. Nothing in Hope indicates that such aggregation would be a problem **in the context of the Hope reference**, nor has the Examiner offered any reasoning as to why a person skilled in the art would anticipate the existence of such problems **in compositions such as those of Hope** . The Examiner must find

motivation in the context of the art, not in the abstract, in order to avoid the unreasoned selection of elements. Here that has not been done. Accordingly, there is no motivation in the cited art to include PEG-lipids in the compositions of Hope. Applicants note that the Examiner asserts that "aggregation will be a problem whether recognized by Hope or not," but has not said why this would be the case in uncharged liposomes with uncharged materials encapsulated therein. There is no evidence of record that aggregation is a problem known to be an issue for all liposomes lacking a PEG lipid, regardless of their chemical makeup.

Applicants also point out once again that the claims call for an intermediate of modified lipid that prevents aggregation without completely eliminating it. This intermediate amount is important to the success of the method of the present invention but is not suggested in the references. Nor is there any indication in the cited references that this would be a result-affecting parameter. As noted in the application (Page 13 and Example 7), the formation of the particles of the invention appears to involve an aggregation step, i.e. it is a structural organization process and not a simple permeability effect as in Hope, and is not a process of simple passage of the charged material, such as a polynucleotide, through the membrane. (Hope, ¶ 17). Using an amount of modified ligand that permits some aggregation, however, is not suggested by the references, nor has the Examiner ever argued that it is. Thus, this element of claim 13 is neither taught nor obvious from the teachings cited by the Examiner.

Finally, Applicants point out that the method of the invention provide advantages that cannot be predicted based on the art. The ability to introduce charged therapeutic agents into a pre-formed lipid particle, without rearrangement is important, because the size of the resulting particle is predictable because it in essence remains unchanged. The size of a particle containing a therapeutic agent can be a significant factor in toxicity and clearance rate of the particles. Thus, the ability to control it predictably is important. This control is lacking in Wheeler, as Wheeler restructures the lipid material (which is not a liposome). In a manufacturing process using the current method, one can add material to the interior of the liposomes without changing the liposome. (Hope, ¶ 14).

The Claims Are Patentable over Hope in view of Malone and Zalipsky

The Examiner rejected claims 13-32 under 35 USC § 103 as obvious over the combination of US Patent No. 6,447,800 of Hope in view of Malone and US Patent No. 6,365,179 of Zalipsky. This rejection is essentially identical to the rejection previously discussed, with Malone filling the role of teaching transfection with charged lipid-oligonucleotide complexes and Zalipsky providing a teaching of modified lipids. This rejection is defective for the same reasons.

Unlike Wheeler, Malone does not specifically discuss the association of the lipids and the nucleic acids on a structural basis. Since the liposomes of Malone are preformed, however, and the RNA is merely added, the reasonable conclusion is the formation of a complex, and not any passage through the lipid membrane to form encapsulated nucleic acid. Thus, Malone says nothing that would provide guidance to justify the combination of references.

The Claims Are Patentable over Schubert in view of Malone and either Zalipsky or WO 98/51278.

The Examiner rejected claims 13-20 and 25-32 under 35 USC § 103 as obvious over the combination of Schubert in view of Malone and either US Patent No. 6,365,179 of Zalipsky or WO 98/51.

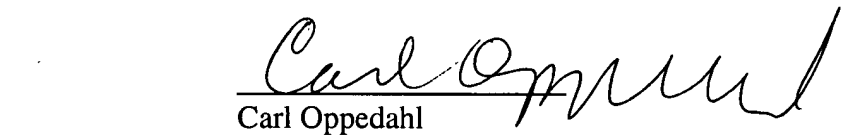
Schubert is relied upon for many of the same teachings as Hope, but is actually less similar to the claimed invention because in Schubert a different method for opening the membrane of a pre-formed liposome is disclosed. Specifically, in the case of Schubert, sodium cholate, a bile salt, is used to open the membrane. The Examiner has not indicated why a person skilled in the art would anticipate that changing the liposome structure to include a cationic lipid would allow loading of pre-formed lipids with a negatively charged oligonucleotide, without this step of membrane opening being necessary. In this regard, it is noted that the cholate part of the bile salt has a negative charge. There is no basis provided in the reference or the Examiner's argument to conclude that if one introduced a cationic lipid into the liposome of Schubert, the

cholate would still function to open the membrane. To the contrary, it might well associate with the positive charges of the cationic lipid, yielding a wholly different result. Thus, it is naive and overly simplistic to argue, as the Examiner has done, that cholate makes holes, and cationic lipids allow transfection, and that we can put the two features together in a single species without concern or thought for the interactions of the cholate and the cationic lipid. Further, the reliance on Malone or Zalipsky in this rejection is flawed for the same reasons as discussed above.

Conclusion

For the reasons set forth above, Applicants submit that the rejections of the claims are in error and should be reversed. Favorable action is respectfully requested.

Respectfully submitted,



Carl Oppedahl
PTO Reg. No. 32,746
Attorney for Applicant
(970) 468-6600

APPENDIX
CLAIMS ON APPEAL

1-12. (canceled)

13. A method for preparing fully lipid-encapsulated therapeutic agent particles of a charged therapeutic agent comprising the steps of

combining a lipid composition comprising preformed lipid vesicles, a charged therapeutic agent, and a destabilizing agent to form a mixture of preformed vesicles and therapeutic agent in a destabilizing solvent, wherein said destabilizing solvent is effective to destabilize the membrane of the preformed lipid vesicles without disrupting the vesicles,

incubating the mixture for a period of time sufficient to allow the encapsulation of the therapeutic agent within the preformed lipid vesicles, and

removing the destabilizing agent,

wherein the preformed lipid vesicles comprise a charged lipid which has a charge which is opposite to the charge of the charged therapeutic agent and a modified lipid having a steric barrier moiety for control of aggregation, and wherein the modified lipid is present in the preformed vesicles in an amount effective to retard, but not prevent, aggregation of the preformed vesicles.

14. The method of claim 13, wherein the charged lipid in the preformed lipid vesicles comprises a cationic lipid and the therapeutic agent is an anionic therapeutic agent.

15. The method of claim 14, wherein the cationic lipid is selected from the group consisting of

dioleyl-N,N-dimethylammonium chloride ("DODAC");

N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTMA");

N,N-distearyl-N,N-dimethylammonium bromide ("DDAB"); N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTAP");

3β-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol ("DC-Chol");

N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide ("DMRIE");

cationic liposomes comprising DOTMA and 1,2-dioleoyl-sn-3-phosphoethanolamine ("DOPE");

cationic liposomes comprising N-(1-(2,3-dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate ("DOSPA") and DOPE;

cationic lipids comprising dioctadecylamidoglycyl carboxyspermine ("DOGS") in ethanol;

N-(2,3-dioleyloxy)propyl)-N,N-dimethylammonium chloride ("DODMA") and

1,2-Dioleoyl-3-dimethylammonium-propane ("DODAP").

16. The method of claim 14, wherein the therapeutic agent is a polynucleotide.

17. The method of claim 16, wherein the cationic lipid is selected from the group consisting of

dioleyl-N,N-dimethylammonium chloride ("DODAC");
N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTMA");
N,N-distearyl-N,N-dimethylammonium bromide ("DDAB"); N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTAP");
3β-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol ("DC-Chol");
N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide ("DMRIE");
cationic liposomes comprising DOTMA and 1,2-dioleoyl-sn-3-phosphoethanolamine ("DOPE");
cationic liposomes comprising N-(1-(2,3-dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate ("DOSPA") and DOPE;
cationic lipids comprising dioctadecylamidoglycyl carboxyspermine ("DOGS") in ethanol;
N-(2,3-dioleyloxy)propyl)-N,N-dimethylammonium chloride ("DODMA") and
1,2-Dioleoyl-3-dimethylammonium-propane ("DODAP").

18. The method of claim16, wherein the lipid composition comprises 10 to 40 mol % of the charged lipid, 25 to 40 mol % of a neutral lipid; 35 to 55 mol % of a sterol, and 2.5 to 10 mol % of the modified lipid.

19. The method of claim 14, wherein the lipid composition comprises 10 to 40 mol % of the charged lipid, 25 to 40 mol % of a neutral lipid; 35 to 55 mol % of a sterol, and 2.5 to 10 mol % of the modified lipid.

20. The method of claim 13, wherein the lipid composition comprises 10 to 40 mol % of the charged lipid, 25 to 40 mol % of a neutral lipid; 35 to 55 mol % of a sterol, and 2.5 to 10 mol % of the modified lipid.

21. The method of claim 13, wherein the destabilizing agent is ethanol.

22. The method of claim 13, wherein the ethanol is present in the destabilizing solvent at a concentration of 25-40 %.

23. The method of claim 22, wherein the cationic lipid is selected from the group consisting of

dioleyl-N,N-dimethylammonium chloride ("DODAC");

N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTMA");

N,N-distearyl-N,N-dimethylammonium bromide ("DDAB"); N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTAP");

3β-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol ("DC-Chol");

N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide ("DMRIE");

cationic liposomes comprising DOTMA and 1,2-dioleoyl-sn-3-phosphoethanolamine ("DOPE");

cationic liposomes comprising N-(1-(2,3-dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate ("DOSPA") and DOPE;

cationic lipids comprising dioctadecylamidoglycyl carboxyspermine ("DOGS") in ethanol;

N-(2,3-dioleyloxy)propyl)-N,N-dimethylammonium chloride ("DODMA") and

1,2-Dioleoyl-3-dimethylammonium-propane ("DODAP").

24. The method of claim 22, wherein the destabilizing solvent further comprises 25 - 300 mM citrate buffer.

25. The method of claim 13, wherein the destabilizing agent is a detergent.

26. The method of claim 25, wherein the cationic lipid is selected from the group consisting of

dioleyl-N,N-dimethylammonium chloride ("DODAC");

N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTMA");

N,N-distearyl-N,N-dimethylammonium bromide ("DDAB"); N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTAP");

3β-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol ("DC-Chol");

N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide ("DMRIE");

cationic liposomes comprising DOTMA and 1,2-dioleoyl-sn-3-phosphoethanolamine ("DOPE");

cationic liposomes comprising N-(1-(2,3-dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate ("DOSPA") and DOPE;

cationic lipids comprising dioctadecylamidoglycyl carboxyspermine ("DOGS") in ethanol;

N-(2,3-dioleyloxy)propyl)-N,N-dimethylammonium chloride ("DODMA") and

1,2-Dioleoyl-3-dimethylammonium-propane ("DODAP").

27. The method of claim 25, wherein the destabilizing solvent further comprises 25 - 300 mM citrate buffer.

28. The method of claim 25, wherein the destabilizing solvent comprises 25 - 300 mM citrate buffer.

29. The method of claim 28, wherein the cationic lipid is selected from the group consisting of

dioleyl-N,N-dimethylammonium chloride ("DODAC");
N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTMA");
N,N-distearyl-N,N-dimethylammonium bromide ("DDAB"); N-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride ("DOTAP");
3β-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol ("DC-Chol");
N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide ("DMRIE");
cationic liposomes comprising DOTMA and 1,2-dioleoyl-sn-3-phosphoethanolamine ("DOPE");
cationic liposomes comprising N-(1-(2,3-dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethylammonium trifluoroacetate ("DOSPA") and DOPE;
cationic lipids comprising dioctadecylamidoglycyl carboxyspermine ("DOGS") in ethanol;
N-(2,3-dioleyloxy)propyl)-N,N-dimethylammonium chloride ("DODMA") and
1,2-Dioleoyl-3-dimethylammonium-propane ("DODAP").

30. The method of claim 13, wherein the mixture is incubated at a temperature of about 40°C.

31. The method of claim 13, wherein the modified lipid is PEG-CerC_{14}.

32. The method of claim 13, wherein the preformed lipid vesicles comprise:
a cationic lipid,
a neutral lipid selected from the group consisting of DOPE and DSPC;
the modified lipid, and
cholesterol.